AMENDMENTS TO THE SPECIFICATION

In the Specification:

In addition to the amendments made in the Preliminary Amendment and in the Supplemental Preliminary Amendment, please make the amendments described below.

Please replace the paragraph on page 10, lines 19-24, with the following paragraph:

--Figure 11 is a representation of current recordings obtained from an ~~oocytes~~oocyte bathed in solutions containing either Ba^{2+} or Ca^{2+} ions. The current responses were induced by co-application of 10 ~~mM~~ μM glycine and 80 ~~mM~~ μM NMDA (single bars). Double bars indicate the co-application of 100 ~~mM~~ μM PS in addition to NMDA and glycine.--

Please replace the paragraph from page 10, line 25, to page 11, line 2, with the following paragraph:

--Figure 12 contains graphical representations of PS dose-responses for NR1/NR2A receptors. Presented are normalized current responses obtained from oocytes injected with (A): $NR1_{000}$ /NR2A, $NR1_{100}$ /NR2A; (B): $NR1_{001}$ /NR2A, $NR1_{101}$ /NR2A; (C): $NR1_{011}$ /NR2A, $NR1_{111}$,/NR2A mRNAs. The current was induced by coapplication of 10 ~~mM~~ μM glycine and 50 ~~mM~~ μM NMDA (for N-terminal insert lacking NR1 isoforms, ~~open~~ closed symbols) or 80 ~~mM~~ μM NMDA (for N-terminal insert containing NR1 isoforms, ~~closed~~ open symbols) and different concentration of PS. Error bars are S.E.M. Solid lines are drawn using equation 1+E max/(1+(EC_{50} /c)n with parameters from Table 3.--

Please replace the previously amended paragraph on page 11, lines 3-13, with the following paragraph:

--Figure 13 ~~contains graphical representations of data which compares 3α5βS and PS dose responses for NR1/NR2A receptors. Presented are normalized current responses obtained~~

~~from oocytes injected with (A): $NR1_{000}$/NR2A, $NR1_{100}$/NR2A; (B): $NR1_{001}$/NR2A, $NR1_{101}$/NR2A; (C): $NR1_{011}$/NR2A, $NR1_{111}$/NR2A mRNAs. The current was induced by coapplications of 10 mM glycine and 50 mM NMDA (for N-terminal insert lacking NR1 isoforms, open symbols or 80 mM (in B; 100 mM in A) NMDA (for N-terminal insert containing NR1 isoforms, closed symbols) and different concentration of 3a5bS (A) or PS (B).~~ contains graphical representations of PS dose-responses for NR1/NR2B receptors. Presented are normalized current responses obtained from oocytes injected with (A): $NR1_{000}$ /NR2B, $NR1_{100}$ /NR2B; (B): $NR1_{001}$ /NR2B, $NR1_{101}$ /NR2B; (C): $NR1_{011}$ /NR2B, $NR1_{111}$,/NR2B mRNAs. The current was induced by coapplication of 10 μM glycine and 50 μM NMDA (for N-terminal insert lacking NR1 isoforms, closed symbols) or 80 μM NMDA (for N-terminal insert containing NR1 isoforms, open symbols) and different concentration of PS. Error bars are S.E.M. Solid lines are drawn using equation $1+E_{max}/(1+(EC_{50}/c)^n$ with parameters from Table ~~2 and~~ 3.--

Please replace the paragraph at page 11, lines 14-23, with the following paragraph:

--Figure 14 contains graphical representations of data which compares 3α5βS and PS dose-responses for NR1/NR2A receptors. Presented are normalized current responses obtained from oocytes injected with $NR1_{XXX}$ /NR2A mRNAs. The current was induced by coapplication of 10 μM glycine and ~~50~~80 μM NMDA (for N-terminal insert ~~containingt~~ containing NR1 isoforms, closed symbols) and different concentrations of 3α5βS (A) or PS (B). Error bars are S.E.M. Solid lines are drawn using equation $1+E_{max}/(1+(EC_{50}/c)^n$ with parameters from Table 3 and 4.--

Please replace the paragraph from page 11, line 24, to page 12, line 4, with the following paragraph:

--Figure 15 contains two graphs of data indicating PS differentially modulates $NR1_{011}$ /2A and $NR1_{111}$ /2A splice variants at pH 7.5. Data points are averaged normalized peak NMDA-induced current responses obtained from oocytes injected with (A) $NR1_{011}$ /2A or (B) $NR1_{111}$

/2A mRNAs. Concentration-response data for NMDA (circles) and for NMDA+100 μM PS (squares) were obtained in the presence of 10 μM glycine. Fitted parameters are (A) (control O, n=14), EC_{50} =~~71~~ 71 μM, Emax=1.20, n_H =1.47; (+PS open squares, n=14), EC_{50} =67 μM, Emax=1.79, n_H =1.24; (B) (control closed circles, n=14), EC_{50} =103 μM, Emax=1.33, n_H =1.67; (+PS closed squares, n=22), EC_{50} =89 μM, Emax=2.70, n_H =2.02. The data were normalized relative to the current induced by co-application of 200 μM NMDA and 10 μM glycine to the same oocyte. Error bars represent S.E.M.--

Please replace the paragraph on page 12, lines 5-19, with the following paragraph:

--Figure 16 contains two graphs of data indicating 3α5βS proportionately modulates $NR1_{011}$ /2A and $NR1_{111}$ /2A splice variants at pH 7.5. Data points are averaged normalized peak NMDA-induced current responses obtained from oocytes injected with (A) $NR1_{011}$ /2A or (B) $NR1_{111}$ /2A mRNAs. Concentration-response data for NMDA (circles) and for NMDA+100 μM ~~PS~~ 3α5βS (squares) were obtained in the presence of 10 μM glycine. Fitted parameters are (A) (control O, n =14), EC_{50} =~~71~~ 71 μM, Emax =1.20, n_H =1.47; (+3α5βS open squares, n=6), EC_{50} =120 μM, Emax=0.52, n_H =1.35; (B) (control, closed circle, n=14), EC_{50} =103 μM, Emax=1.33, n_H =1.67; (+3α5βS, closed squares, n=8), EC_{50} =158 μM, Emax=0.54, n_H =1.50. The data were normalized relative to the current induced by co-application of 200 μM NMDA and 10 μM glycine to the same oocyte. Error bars represent S.E.M.--

Please replace the paragraph from page 49, line 20, to page 50, line 13, with the following paragraph:

--Example 3

Figure 11 shows a comparison of traces of NMDA induced responses obtained from oocytes expressing $NR1_{100}$ /NR2A receptors bathed in normal (Ca^{2+} containing) Ringer solution and Ba-Ringer solution in which Ca^{2+} was replaced with Ba^{2+}. Current traces obtained in Ba-Ringer solution do not exhibit the rapidly inactivating component that is seen in normal Ringer,

and which most likely reflects current through Ca^{2+} activated Cl^- channels (Leonard et al., Neuron 4: 53-60 (1990)). All further experiments were performed in Ba-Ringer. The current responses obtained from oocytes injected with NMDA receptors composed of different NR1 isoforms differed from each other in agonist EC_{50} s. L-glutamate, glycine and NMDA EC_{50} s for NR1 isoforms lacking N-terminal insert were less than ones for isoforms with N-terminal insert (except for $NR1_{001}$, see Table 2). The absolute current responses induced by saturating concentration of agonists (500 ~~mM~~ μM NMDA and 10 ~~mM~~ μM glycine) were in the range from 800 to 1800 nA. Since a steroid's effect on NMDA receptors was found to depend on level of expression of the receptor in the membrane of oocytes (see below), oocytes that showed current responses in the range of 0.1 μAmp to 1 μAmp induced by 50 ~~mM~~ μM (or 80 ~~mM~~ μM NMDA for isoforms with N-terminal insert) and 10 ~~mM~~ μM glycine were used to assess the effects of pregnenolone sulfate and pregnanolone sulfate on NMDA receptors comprised of different isoforms of NR1 subunit.--

Please replace the paragraph on page 50, lines 14-22, with the following paragraph:

--Co-application of 100 ~~mM~~ μM PS resulted in potentiation of current through NMDA receptors comprising any NR1 isoform. NMDA, L-glutamate and glycine dose-response experiments showed that potentiation by PS was observed even at saturating concentrations of agonists. In addition, the agonists EC_{50} obtained in the absence of steroid were similar to ones obtained in the presence of 100 ~~mM~~ μM PS, suggesting that PS did not compete for the agonists binding sites (see Table 2).--

Please replace the table on pages 51-53 (Table 2) with the following table:

Subunits	NMDA (PS) EC_{50}, [[mM]] μM	n_{Hill}	E_{max}

$NR1_{011}$/NR2A	52±6.5 (43±4.8) n=4 (n=4)	1.39±0.07 (1.11±0.12)	1.14±0.02 (1.68±0.06)
$NR1_{111}$/NR2A	88±16 (95±12) n=4 (n=4)	1.49±0.02 (1.33±0.22)	1.34±0.09 (2.27±0.07)
$NR1_{001}$/NR2A	81±7 (71±18) n=8 (n=4)	1.46±0.07 (1.21±0.15)	1.27±0.04 (2.15±0.17)
$NR1_{010}$/NR2A	44±8 (22±2) n=7 (n=4)	1.19±0.06 (1.13±0.09)	1.18±0.03 (1.53±0.08)
$NR1_{000}$/NR2A	60±7 (33±3) n=7 (n=11)	1.31±0.08 (1.18±0.05)	1.20±0.05 (1.55±0.04)
$NR1_{101}$/NR2A	75±6 (35±5) n=5 (n=4)	1.31±0.05 (1.15±0.12)	1.33±0.07 (1.78±0.06)
$NR1_{100}$/NR2A	76±6.2 (40±3.3) n=11 (n=6)	1.13±0.04 (1.20±0.06)	1.36±0.04 (1.60±0.07)

L-glutamate (PS)

$NR1_{011}$/NR2A	0.56±0.03 n=4	(0.39±0.03) (n=4)	1.17±0.03	(1.13±0.08	1.59±0.03	(2.08±0.21)
$NR1_{111}$/NR2A	0.71±0.06 n=4	(0.51±0.03) (n=4)	1.39±0.02	(1.58±0.09)	1.87±0.12	(2.62±0.27)
$NR1_{001}$/NR2A	1.03±0.09 n=7	(0.65±0.21) (n=4)	1.20±0.07	(1.57±0.18)	2.10±0.12	(2.85±.26)
$NR1_{010}$/NR2A	0.38±0.04 n=9	(0.25±0.01) (n=4)	1.32±0.14	(1.59±0.15)	1.48±0.03	(1.81±0.08)
$NR1_{000}$/NR2A	0.65±0.03 n=4	(0.59±0.04) (n=4)	1.05±0.02	(1.17±0.08)	1.75±0.02	(2.92±0.03)
$NR1_{101}$/NR2A	0.68±0.06 n=3	(0.60±0.04) (n=4)	1.07±0.06	(1.37±0.06)	1.77±0.09	(2.72±0.07)
$NR1_{100}$/NR2A	0.45±0.02 n=4	(0.31±0.02) (n=4)	1.15±0.04	(0.98±0.06)	1.50±0.04	(2.30±0.21)

glycine (PS)

$NR1_{011}$/NR2A	0.92±0.11	(0.85±0.1)	1.30±0.05	(1.34±0.18)	1.67±0.08	(2.24±0.29)

	n=4	(n=3)				
$NR1_{111}$/NR2A	1.22±0.09 n=4	(1.07±0.09) (n=8)	1.49±0.06	(1.26±0.10)	1.93±0.07	(2.68±0.16)
$NR1_{001}$/NR2A	1.79±0.3 n=6	(1.56±0.65) (n=4)	1.25±0.07	(1.30±0.13)	1.89±0.20	(2.77±0.40)
$NR1_{010}$/NR2A	0.90±0.08 n=4	(1.07±0.11) (n=4)	1.32±0.07	(1.38±0.10)	1.51±0.07	(1.92±0.19)
$NR1_{000}$/NR2A	0.97±0.10 n=3	(1.46±0.17) (n=4)	0.90±0.01	(1.12±0.05)	1.48±0.04	(2.88±0.27)
$NR1_{101}$/NR2A	1.74±0.26 n=5	(1.07±0.17) (n=4)	1.37±0.13	(1.32±0.09)	1.63±0.04	(2.46±0.23)
$NR1_{100}$/NR2A	0.82±0.11 n=4	(0.97±0.07) (n=4)	1.23±0.07	(1.03±0.05)	1.52±0.07	(2.34±0.09)

Table 2. Properties of NMDA receptors comprising different NR1 isoforms in the absence and in the presence of 100 mM PS.

Please replace the paragraph on page 54, lines 1-19, with the following paragraph:

--In order to construct PS dose-response curves different concentrations of PS (in the range from 0.5 to 400 ~~mM~~ μM) were coapplied with 10 ~~mM~~ μM glycine and 50 ~~mM~~ μM (or 80 ~~mM~~ μM NMDA, which is close to EC_{50} values for NR1 isoforms without or with N-terminal insert respectively). The dose-response experiments revealed that PS had similar potency for all combinations of $NR1_{XXX}$ and NR2A subunits. However, PS was more efficient when applied to NMDA receptors containing NR1 isoforms that were lacking N-terminal insert (see Figure 12). Maximum potentiation of NMDA-induced current for $NR1_{111}$ /, $NR1_{101}$ /, $NR1_{100}$ /NR2A receptors were 1.51.+-.0.04 (n=9), 1.66.+-.0.10 (n=8), 1.67.+-.0.07 (n=8) fold respectively in comparison to 2.14.+-.0.17 (n=7), 2.19.+-.0.09 (n=9), 2.14.+-.0.08 (n=15) fold potentiation for $NR1_{011}$ /, $NR1_{001}$ /, $NR1_{000}$ /NR2A receptors respectively (see Table 3). T-test analysis of maximum PS potentiation obtained from receptors comprising NR1 isoforms with and without

N-terminal insert resulted in p values of 0.024, 0.005, 0.002 for pairs 111 vs. 011, 101 vs. 001 and 100 vs. 000 respectively.--

Please replace the table on page 55 (Table 3) with the following table:

	--EC_{50}, ~~mM~~ μM	n_{Hill}	E_{max}
$NR1_{111}$ /NR2A	40 ± 8 (n = 9)	1.28 ± 0.19	0.51 ± 0.04
$NR1_{011}$ /NR2A	32 ± 8 (n = 7)	1.18 ± 0.18	1.14 ± 0.17
$NR1_{101}$ /NR2A	25 ± 5 (n = 8)	1.52 ± 0.24	0.66 ± 0.10
$NR1_{001}$ /NR2A	26 ± 2 (n = 9)	1.14 ± 0.10	1.19 ± 0.09
$NR1_{100}$ /NR2A	24 ± 5 (n = 8)	1.33 ± 0.08	0.67 ± 0.07
$NR1_{000}$ /NR2A	29 ± 3 (n = 15)	1.31 ± 0.06	1.14 ± 0.08
$NR1_{010}$ /NR2A	48 ± 12 (n = 5)	1.30 ± 0.18	1.03 ± 0.09
$NR1_{111}$ /NR2B	24 ± 1 (n = 3)	1.52 ± 0.10	1.20 ± 0.10
$NR1_{011}$ /NR2B	35 ± 10 (n = 4)	1.29 ± 0.19	0.78 ± 0.14
$NR1_{101}$ /NR2B	32 ± 8 (n = 2)	1.63 ± 0.29	1.03 ± 0.02
$NR1_{001}$ /NR2B	24 ± 6 (n = 4)	1.34 ± 0.17	0.63 ± 0.07
$NR1_{100}$ /NR2B	34 ± 4 (n = 4)	1.42 ± 0.11	1.02 ± 0.10
$NR1_{000}$ /NR2B	22 ± 7 (n = 4)	1.52 ± 0.15	0.58 ± 0.04

Table 3. Effect of PS on NMDA receptors comprising different NR1 isoforms.--

Please replace the paragraph on page 55, lines 1-11, with the following paragraph:

--In contrast to potentiating effect of PS, 3α5βS induced current inhibition through receptors comprising any NR1 isoform (see ~~Figure 13~~Figure 14A). The inhibitory effect was reversible and concentration dependent. The potency of 3α5βS obtained for NMDA receptors comprising different NR1 isoforms were similar, revealing ~~PS~~ 3α5βS EC_{50} s that ranged from 25 to 45 ~~mM~~ μM. The maximum extent of inhibition obtained for different NR1 isoforms ranged from 75±7% (for $NR1_{111}$ /NR2A) to 99±3% (for $NR1_{100}$ /NR2A, see Table 4). The differences in maximum inhibition were insignificant and no apparent correlation with the presence of N-terminal insert was observed.--

Please replace the table on page 56 (Table 4) with the following table:

--Splice variant	EC_{50}, ~~mM~~ μM	n_{Hill}	E_{max}
111	29 ± 5 (n = 4)	1.09 ± 0.9	-0.75 ± 0.07
011	38 ± 0.5 (n = 4)	0.96 ± 0.06	-0.86 ± 0.04
101	45 ± 2 (n = 3)	1.05 ± 0.25	-0.90 ± 0.07
001	25 ± 2 (n = 4)	0.95 ± 0.11	-0.89 ± 0.05
100	36 ± 2 (n = 4)	0.83 ± 0.02	-0.99 ± 0.03
000	41 ± 0.6 (n = 4)	0.98 ± .03	-0.89 ± 0.01
010	34 ± 4 (n = 4)	1.04 ± 0.06	-0.85 ± 0.02

Table 4. Effect of 3α5βS on NMDA receptors comprising different NR1 isoforms.--